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STUDIES ON TRANSMISSION OF LEUKEMIA IN RATS

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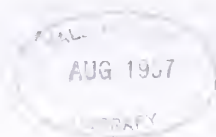
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INTRODUCTION

Although a considerable amount of investigation has been concerned with the transmission of tumors and leukemias from one animal to another, it has been noted that most of the work with transmissible leukemia has been done with mice, and comparatively little with rats. Also noted was the fact that these studies in the mouse, particularly in the past few years, have concentrated on the use of cell-free filtrates of leukemic material, which have been shown to produce leukemia upon injection into the recipient mice. The rat studies have involved the use of carcinogenic agents to produce leukemia, and the transplantation of the induced leukemia by injection of leukemic tissue.

Since previous studies in the transmission of rat leukemia have involved only the injection of leukemic material, the question arose as to the possibility of other means of transmission, especially in the light of discoveries which strongly suggest a viral etiology, at least in mice, in whom transmission occurs without the use of particulate matter. Therefore, it was decided to make an attempt to transfer rat leukemia via the lungs and the gastro-intestinal tract, and to see if radiation and the use of hydrocortisone had any effect on the transmissibility by these routes.

The rats used in this study were inbred albino rats of the WR strain, in whom an acute lymphocytic leukemia was developed (Furth). When cell suspensions from leukemic WR rats are in-

jected into normal adult rats of the same strain, there is virtually one hundred per cent transmission of leukemia to the recipient animals.

During the course of the experiment, a group of Sprague-Dawley rats instead of WR rats was irradiated by accident. It was therefore decided to use these as recipient animals for intraperitoneal injections of leukemic cells from the WR rats, to see if it were possible to transmit this leukemia to another strain, both with and without the use of irradiation.

HISTORICAL REVIEW

In recent years, the induction and transfer of leukemia in animals, particularly mice and rats, has gained much interest. While spontaneous leukemia is quite rare in the rat, it has been found in mice. The AK strain of mice, which has a high incidence of spontaneous leukemia, has been used extensively in transmission experiments. Furth, in 1935, was the first to describe the transfer of myelogenous leukemia in mice (1). In 1937, Furth and Kahn succeeded in transferring mouse leukemia by injection of a single cell, but not by cell-free extracts (2). Much work has been done by Gross, using the AK strain. He reported the transmission of leukemia by inoculation of cell suspensions from leukemic AK mice into newborn C3H mice, which have a low incidence of spontaneous leukemia (3). Stewart obtained similar results (4). Gross was also able to transmit the AK leukemia to the C57 strain, and noted that susceptibility of the C3H and C57 mice to the transplanted leukemia declined markedly after the first week of life (5).

Although chicken lymphomatosis was transmitted by Bang and Ellerman in 1908 by the use of cell-free extracts (6), it was not until Gross in 1951 reported the transmission of mouse leukemia by injection of cell-free extracts (7), that any interest was evinced in this method of transferring mouse leukemia. Since that time, numerous experiments have been performed by Gross and others, using cell-free filtrates of

leukemic mouse organs.

Comparatively little work has been done in the field of rat leukemia. Before 1936, leukemia had not yet been reported in any strain of rat. In that year Wilens and Sproul described myelogenous and lymphatic leukemia in a group of Osborne-Mendel rats, Oberling, Guérin, and Guérin, in 1939, also described several cases of leukemia among a group of rats, and Arai, in 1940, found a case of myelogenous leukemia in a large colony of rats among which 27 had developed spontaneous tumors (8). Spontaneous leukemia occurs rarely in Wistar rats, according to Gruenstein and Shay (9), and Shay et al. have noted that spontaneous myelogenous leukemia has never been reported in this strain (8).

The induction of both myelogenous and lymphatic leukemia in rats by the use of carcinogenic agents has been reported by several investigators. Murphy and Sturm were the first to report transplantable lymphatic leukemia, induced by the injection of a Wistar rat with 1,2,5,6-dibenzanthracene. When leukemic cells were injected intraperitoneally into other Wistar rats, the recipients developed lymphatic leukemia with involvement of the lymph nodes and thymus. Subcutaneous injection produced local lymphosarcomas with involvement of local lymph nodes, and inoculation into the spleen produced leukemia with involvement of the spleen and liver (10).

Shay et al. reported the development of several cases of lymphatic and myelogenous leukemia among a group of Wistar rats

following the gastric instillation of methylcholanthrene over a period of 1 to 14 months (11). These investigators have been able to transfer both types of leukemia to normal Wistar rats and also to rats of the Sherman and Long-Evans strains (8). They found that successful transfer occurred in a very high percentage of cases when recipients 1 to 7 days old were used, and that the number of successful transfers declined sharply with age, a phenomenon noted by Gross working with the transmission of mouse leukemia (5). The methods used for transfer were subcutaneous and intraperitoneal injection of whole blood, and spleen, liver, lymph nodes, and thymus (9). It was found that the virulence of the leukemia increased on repeated transfer, as it has been found to do in mice (12).

Furth has developed an acute lymphocytic leukemia in an inbred Wistar strain of albino rats. This leukemia is transmissible to adult rats of the same strain, and has been transplanted by the use of intramuscular and intraperitoneal injection of blood and organs from leukemic animals. Leukemia develops in the host animals in 10 to 14 days, and the period of survival varies from 10 to 30 days. It has been found on repeated transfer, the leukemia has become more virulent, as is the case with other transmissible leukemias. The advantages of using the WR leukemia for these studies are its high degree of transmissibility and its ability to infect adult recipients.

It has been established that while small doses of irradi-

iation may enhance immunity, large doses will decrease the immune response (13). This has been demonstrated in various mammals, in whom there has been found lowered resistance to induced infections and to natural inhabitants of the intestine, after large doses of total body irradiation. It is possible to transmit leukemia to resistant strains of mice by prior irradiation (14),(15), and the transmission of mouse leukemia to rats has been accomplished by the use of X-radiation (16). Hektoen, Dixon et al., and others have found that the inhibitory effect of radiation on the antibody response depends on radiation-induced changes which occur prior to the administration of antigen, and that radiation has little effect on antibodies already formed (17),(18), Kohn has shown a striking reduction in the antibody response to injected sheep cells in rats previously irradiated with 350r. When radiation was delivered several days after injection the dose had to be doubled in order to obtain the same reduction in antibodies, and the reduction was found to be transient (19).

The mechanism by which radiation decreases immunity has been explained on the basis of several factors: injury to the phagocytic mechanism, decreased formation of antibodies, and destruction of lymphocytes (13).

There is much conflicting evidence in regard to the relationship between adrenal cortical hormones and antibody production. Some investigators have claimed that adrenal cortical hormones stimulate the release of antibodies, while others have

observed no effect on antibody levels. However, most of the evidence now seems to be in accord with the theory that these hormones depress antibody formation. Berglund has shown that a significant reduction in antibody formation is produced by cortisone administration before injection of antigen, and that no significant change in antibodies is found if cortisone is administered after antigen injection (20). Cortisone was also found to decrease antibody formation in vitro (21). Hydrocortisone has been shown to be capable of reversing the electrical charge change wrought on the red cell by antibody action in vitro (22). Taking advantage of this property of cortisone, Werder et al. were able to transplant leukemia to a previously resistant strain of mice by prior injection of cortisone (14). Similarly, Braunsteiner and Pakesch observed the growth of human leukemic cells in rats previously treated with cortisone and nitrogen mustard (23). However, in contrast with these results, Murphy and Sturm found that adrenalectomy increased the susceptibility of rats to a transplantable lymphatic leukemia and that the administration of ACTH and cortical extracts gave protection against leukemia in a highly susceptible strain of rats (24), (25).

In view of the results obtained by previous investigators working with cortisone and irradiation, it was felt that the use of these methods of reducing resistance might facilitate the transmission of leukemia in rats.

EXPERIMENTAL PROCEDURE

EXPERIMENT I. ATTEMPTS AT TRANSMISSION OF WR LEUKEMIA VIA THE GASTRO-INTESTINAL TRACT AND LUNGS.

A. Preparation of leukemic organ homogenate

In order to obtain the leukemic material, leukemic WR rats, their total white blood counts averaging about 300,000 wbc/mm³, were sacrificed. Each was anesthetised with 0.1 to 0.2 cc. of Nembutal and its abdomen opened; about 6 cc of blood was withdrawn by intercardiac puncture through the diaphragm. Then the liver and spleen were removed, cut into small pieces, and squeezed through a garlic press. A small amount of normal saline was added, and the mixture was strained through gauze to remove any strands of connective tissue or large pieces of material. The oxalated blood was then added to the organ homogenate. In each case, the homogenate was used within an hour of its preparation.

B. Transmission via inhalation

In this experiment, 12 normal adult WR rats were used. Four of these were irradiated with 300r 3 days before inhalation of leukemic material and 4 were injected intraperitoneally with Solu-corter (hydrocortisone sodium succinate) 10 mg/kilo, on two successive days, three days before inhalation. The other 4 animals received no pre-treatment.

For the purposed of exposing the animals to a fine mist of the leukemic organ homogenate, so that they might inhale it

for several hours, a few cc. of the mixture were placed in a glass nebulizer which was connected by rubber tubing to an air jet. The nebulizer was taped to the rat cage, which was placed in a clear plastic bag, the bag was tied securely around the tubing so that it was airtight save for the inlet tubing from the air jet to the nebulizer and an outlet tube leading into a container of water. When the air jet was turned on, a fine mist was produced, and this was maintained for a period of about 2 hours.

Total white blood counts were performed before, and then 3 weeks after the animals were treated.

C. Transmission via the gastro-intestinal tract

Eleven normal adult WR rats were used. Four animals were pre-treated with hydro-cortisone and four were irradiated with 300r, in the same manner as described in section B. Three other rats received no treatment previous to feeding with the leukemic organ homogenate.

In order to feed the leukemic homogenate, the rats were intubated, and the material injected from a syringe into the feeding tube (26). The animal was laid on its side and held down with the left hand. The left thumb was placed at the angle of the jaw in order to pull open the mouth. A #8 soft rubber French catheter, moistened with water, was then introduced into the stomach with the right hand, and 2 cc. of the leukemic organ homogenate were injected.

Total white blood counts were performed before treat-

ment, and then 3 weeks afterward.

EXPERIMENT II. CROSS-STRAIN TRANSMISSION OF WR LEUKEMIA

Fourteen normal adult Sprague-Dawley rats were used in this experiment. Each animal was injected intraperitoneally with about 3 cc. of the leukemic organ homogenate. Eight rats were irradiated with 300r 6 days prior to injection, and the other group of 6 animals received no pre-treatment. Total white blood counts were done before injection and then at 3 and 4 weeks after injection.

EXPERIMENTAL RESULTS

EXPERIMENT I. ATTEMPTS AT TRANSMISSION OF LEUKEMIA TO WR RATS VIA THE LUNGS AND GASTRO-INTESTINAL TRACT.

A. Transmission via inhalation

The results of this experiment, as seen in table I, show that the 12 WR rats which were exposed to a fine mist of the leukemic homogenate did not develop leukemia. The total white blood count of all the animals in each of the 3 groups (control, irradiated and hydrocortisone-treated) remained essentially the same at 3 weeks and their smears showed no leukemic cells. All the animals in each of the 3 groups were alive and showed no signs of leukemia after nearly 2 months, except for one in the irradiated group and one in the hydrocortisone-treated group, who died during the fourth week after treatment. Death occurred without either of the 2 rats showing any evidence of leukemia. Unfortunately, these were not autopsied and the cause of death was not ascertained.

TABLE I

TOTAL LEUKOCYTE COUNTS* OF WR RATS TREATED BY INHALATION OF
LEUKEMIC HOMOGENATE

A. Controls (no pre-treatment)

#	normal	3 weeks
1	6,600	5,050
2	8,850	8,100
3	12,500	15,450
4	9,800	16,250

B. Pre-treated with 300r

#	normal	3 weeks	
1	19,600	18,550	
2	12,800	13,750	Died
3	13,950	14,000	
4	13,500	12,650	

c. Pre-treated with hydrocortisone

#	normal	3 weeks	
1	13,050	12,900	
2	16,750	16,850	
3	14,000	13,650	
4	11,350	12,500	Died

* wbc/mm³

B. Transmission via the gastro-intestinal tract

Of the 11 WR rats that were intubated and fed the leukemic organ mixture, none in any group (control, irradiated, and hydrocortisone-treated) developed leukemia. Their total white counts remained normal and their smears showed no abnormal cells at 3 weeks. All animals were alive and healthy 2 months after treatment.

TABLE II

TOTAL LEUKOCYTE COUNTS* OF WR RATS TREATED BY FEEDING OF LEUKEMIC HOMOGENATE

A. Controls (no pre-treatment)

#	Normal	3 weeks
1	21,950	16,750
2	17,650	17,050
3	13,050	15,500

B. Pre-treated with 300r

#	Normal	3 weeks
1	11,650	11,050
2	12,050	13,400
3	14,300	14,000
4	12,450	14,600

c. Pre-treatment with hydrocortisone

#	normal	3 weeks
1	13,900	15,050
2	15,150	16,100
3	12,400	11,250
4	13,350	14,500

EXPERIMENT II. CROSS-STRAIN TRANSMISSION OF WR LEUKEMIA

Fourteen normal adult Sprague-Dawley rats were given intraperitoneal injections of leukemic organ homogenates obtained from leukemic rats. Group A received no treatment before injection and Group B received 300r 6 days prior to injection.

A. Untreated rats

In Group a the pre-injection total white counts ranged between 22,000 and 30,150 wbc/mm³. Counts done 3 weeks after the injection of leukemic material showed no significant change, nor did the smears reveal any leukemic cells. However, two of the animals, (#5 & 6), appeared sick and died between the third and fourth week after injection. Counts repeated at four weeks on the 4 remaining animals showed little variation in total white count, save for one animal (#3), whose count dropped from 20,050 to 15,150 at 4 weeks. Inspection of a smear showed no abnormal cells. This animal also appeared sick, and subsequently died. At 5 weeks, 3 animals were still alive and appeared healthy. Their total white counts showed little change, and no leukemic cells were seen in the stained smears.

B. Rats pre-treated with 300r

Before irradiation, the total white counts of these rats ranged between 11,650 and 27,600 wbc/mm³. Three weeks after intraperitoneal injection of leukemic homogenate, 2 animals had died and 3 had become leukemic, with total white counts of 250,000, 325,650, and 200,350. Two other animals (#7 & 8) still had normal white counts. #6 was thought to have developed a tumor in the abdominal wall at the site of injection, so it was

sacrificed at this time. However, on inspection, the tumor was found to be an abscess. At 4 weeks the leukemic animals had died, leaving only 2 animals (#7 & 8), whose counts were still normal.

TABLE III

TOTAL LEUKOCYTE COUNTS* OF SPRAGUE-DAWLEY RATS INJECTED INTRA-
PERITONEALLY WITH LEUKEMIC HOMOGENATE.

A. No pre-treatment

#	Normal	3 weeks	4 weeks	5 weeks
1	22,000	21,700	20,200	16,350
2	30,150	29,350	26,250	25,200
3	22,500	20,500	15,150	Died
4	24,200	24,000	23,400	23,050
5	23,750	21,050	Died	-
6	22,350	24,300	Died	-

B. Pre-treated with 300 r

#	Normal	3 weeks	4 weeks
1	11,800	Died	-
2	27,600	250,000	Died
3	11,650	325,650	Died
4	12,250	Died	-
5	19,950	200,350	Died
6	12,550	Sacrificed	-
7	19,600	17,950	14,050
8	20,400	19,900	19,700

* wbc/mm³

DISCUSSION

I-WR STRAIN RATS

It is evident from the observations that no rats in this group developed leukemia when transmission was attempted by feeding or by inhalation of leukemic cells. The use of hydrocortisone and irradiation in order to diminish antibody response did not appear to increase the susceptibility. It was noted that of a group of 23 rats used in this part of the experiment, only 2 died, both of which had been treated by inhalation of leukemic cells, one having been given hydrocortisone previously, and the other having been exposed to 300r. Both animals had had normal total white counts at 3 weeks, with no evidence of leukemic cells in their smears, and they appeared perfectly healthy. Sometime during the fourth week, however, they had disappeared, and it was assumed that they had died and been removed, so that they were not available for autopsy. Since it is probable that these animals would have begun showing signs of leukemia at least a week before death, it is not likely that they died of leukemia, but rather of other causes.

Although cell-free transmission of this leukemia has not been attempted, it is possible that the etiology is viral, as in the AK mouse leukemia, and that transfer may be effected by the use of cell-free filtrates. If this is the case, it would appear likely that transmission through the lungs and gastro-intestinal tract is possible, even though there may be

destruction of the leukemic cells introduced into these organs. On the other hand, local inactivation of the leukemic factor, especially in the gastro-intestinal tract by the action of acid or enzymes, is very likely. Gross has shown that the mouse leukemic factor can be inactivated by heating (27), and by the addition of ether (28). Therefore it seems probable that there are many factors in the body which may render an animal resistant. It was thought that hydrocortisone or irradiation might lower the antibody response and thereby increase the susceptibility to leukemia, but it is evident from the results that other factors are involved as well. It is probable, too, that adrenal cortical hormones and irradiation have other effects on resistance, and that the mechanism whereby the susceptibility of an animal to transplanted leukemia is altered, may not be wholly a function of decreased antibody response.

If the two rats that died of unknown causes did have leukemia, it is worth noting that both these animals had been subjected to previous treatment, one to hydrocortisone and the other to irradiation, and that both had inhaled the leukemic material. This would suggest that lowering of the antibody response had increased their susceptibility, and that of the two methods of transfer, inhalation was the more successful. Lowering of the antibody response may have decreased their resistance to infection, and this may well have been the cause of death. In any event, however, definite conclusions cannot be drawn from so small a series as to the effectiveness of

these methods of transmission.

It would be of great interest to investigate the effectiveness of transfer via the lungs and intestine under conditions in which the integrity of the alveolar membrane and intestinal mucosa had been damaged, such as by the administration of irritants.

II-SPRAGUE-DAWLEY RATS

The results obtained after intraperitoneal injection of WR leukemic cells into Sprague-Sawley rats demonstrate that this leukemia is transmissible to another strain of rats. In the non-irradiated control group, 3 animals of 6 were still alive and showed no evidence of leukemia after 5 weeks. Two other animals died at about 4 weeks and 1 at 5 weeks. These rats appeared sick, but their total white counts were not elevated and their smears showed no leukemic cells. Unfortunately, the cause of death was not ascertained in these animals; even though the blood was normal in all cases within a week of death, there is a possibility that leukemia developed very rapidly and produced death in the space of a week. However, there was no evidence of leukemia in any of the 6 rats at 3 weeks. In contrast to these results, among the group of 8 animals previously irradiated, it was found that 3 weeks after injection of leukemic cells, 2 had died and 3 were definitely leukemic, with counts between 250,000 and 325,650 per cubic millimeter. The three leukemic animals died within the following week. Of the 3 rats alive at 3 weeks, one was sacrific-

iced because it was thought to have a tumor in the abdominal wall, (this was found to be an abscess), and the 2 remaining animals were normal. These were still alive and not leukemic more than 4 weeks after injection. These results suggest that previous irradiation is necessary for the transmission of WR leukemia to Sprague-Dawley rats. If the cause of death in those non-irradiated rats that died was leukemia, then it may be assumed that radiation is not a necessary factor. However, comparison of the results in both groups suggests that although radiation may not be the deciding factor, it hastens the development of leukemia, as it can be seen that all of the non-irradiated group showed no signs of leukemia at 3 weeks after injection, while 2 of the irradiated group had already died and 3 others were definitely leukemic at that time.

It is not too suprising that WR leukemia could be transmitted to a different strain of rats, in the light of previous investigations. Gross, working with mouse leukemia, was able to transmit it from AK mice to the C3H and C57 strains (3), (5). Shay et al, transmitted leukemia induced by methylcholanthrene in Wistar rats to rats of the Sherman and Long-Evans strains (29). Kaalund-Jorgenson, in 1940, reported the transfer of mouse leukemia to rats after irradiation, and Pakesch and Braunsteiner in 1955 observed the growth of human leukemia cells in rats previously treated with nitrogen mustard and cortisone (16),(23).

Gross has shown that when cell suspensions or cell-free

extracts of AK mouse leukemia were inoculated into newborn C3H mice, leukemia developed 2 to 4 weeks later. This leukemia could be grafted back to the AK mice, but not to adult C3H mice. When filtered extracts of AK leukemia were inoculated into C3H newborns, the leukemia which developed in some after $3\frac{1}{2}$ to 27 months, was found to be specific for the recipient strain and could be transplanted to adult C3H mice, but only occasionally back to the AK strain. These results suggested to Gross that the agent causing leukemia in the C3H mice was a cell-free, transmissible agent which acted on the cells of its new host. Experiments using C57 mice yielded similar results (30).

Keeping in mind the theory proposed by Gross, it would be of interest to see whether or not the WR rat leukemia is transmissible to other strains by injection of cell-free filtrates, and to observe the results of grafting the induced WR leukemia in Sprague-Dawley rats back to WR rats and also to other rats of the Sprague-Dawley strain.

SUMMARY

1. Under the conditions of this experiment, it was found that acute lymphocytic leukemia was not transmissible from WR rats to normal rats of the same strain, via the lungs or gastro-intestinal tract.
2. Pre-treatment of WR rats with hydrocortisone and irradiation did not appear to reduce their resistance to infection with leukemia via the lungs or gastro-intestinal tract.
3. Cross-strain transmission of WR leukemia was effected by intraperitoneal injection of leukemic organ homogenates into Sprague-Dawley rats..
4. The results of this study suggest that cross-strain transmission of leukemia is facilitated by pre-treatment of the recipient animals with irradiation.

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